0013-7227/79/1052-0442$02.00/0
Endocrinology

Vol. 105, No. 2
Printed in U.S.A.

Ovarian Responses of Pregnant Mare Serum Gonadotropin- and Human Chorionic Gondotropin-Primed Rats: Desensitizing, Luteolytic, and Ovulatory Effects of a Single Dose of Human Chorionic Gonadotropin*

MARY HUNZICKER-DUNN, SHARON L. DAY, JOEL ABRAMOWITZ, AND LUTZ BIRNBAUMER

Department of Biochemistry, Northwestern University Medical School Chicago, Illinois 60611; and the Department of Cell Biology, Baylor College of Medicine, Houston, Texas 77035

ABSTRACT. We conducted a study to determine the morphological appearance and functional responsiveness of ovarian tissues after administration of hCG to 28-day-old rats primed 65 h earlier with PMS gonadotropin (PMSG) and after administration of a second dose of hCG 5 days later, *i.e.* to 33-day-old rats containing heavily luteinized ovaries. Sixty-five hours after the administration of 50 IU PMSG sc to 25-day-old rats, ovaries already contained an abundance of luteinized follicles and an adenylyl cyclase (AC) system that was responsive to LH, epinephrine, and NaF. The administration of 50 IU hCG sc at this time initially resulted in a loss of LH-responsive ovarian AC. Within 4 days of the hCG injection, the ovaries of the now 32-day-old rats were heavily luteinized, and ovarian AC was highly responsive to LH, epinephrine, and NaF.

The administration of a single sc dose of 200 IU hCG to 33-day-old PMSG- and hCG-primed rats with luteinized ovaries resulted in a rapid desensitization of the ovarian AC to LH and a drop in serum progesterone levels. During the subsequent 7 days, serum progesterone levels continued to decline, while total ovarian AC reacquired responsiveness to LH by days 4–5 after the desensitizing dose of hCG. Dissection of ovarian components revealed, however, that the AC system of the corpora lutea originally present at the time of the second hCG injection remained permanently refractory to LH and that the AC in corpora lutea newly formed from freshly ovulated follicles exhibited a significant responsiveness to LH, epinephrine, and NaF. However, these new corpora lutea were not fully active, since serum progesterone never rose.

Subcutaneous administration of 50 IU hCG to 33-day-old PMSG- and hCG-primed rats also promoted a rapid loss of AC responsiveness to LH. This lower concentration of hCG was not sufficient to promote follicular development or ovulation, and the ovarian AC remained refractory to LH for at least 7 days. Intravenous administration of 75 IU hCG to 33-day-old PMSG- and hCG-primed rats similarly promoted a rapid and permanent loss of luteal AC responsiveness to LH; again, follicles did not mature to a preovulatory state and, in fact, appeared to undergo atresia rather than ovulation.

These results indicate that in heavily luteinized ovaries 1) hCG promotes desensitization of rat luteal AC to LH, 2) desensitization of AC to LH stimulation in corpora lutea is permanent and irreversible, and 3) only under conditions where follicles mature and ovulate and new corpora lutea are formed does total ovarian AC reacquire responsiveness during the subsequent week. (*Endocrinology* **105:** 442, 1979)

ONE OF the earliest events in corpora lutea (CL) that follows after the administration of an ovulatory/luteolytic dose of hCG to pregnant or pseudopregnant rats or rabbits is a loss of responsiveness or desensitization of the highly LH-responsive adenylyl cyclase (AC) system to LH (1–5). In rabbits, it is well known that the effect of hCG on CL function is permanently deleterious. Thus, within 24 h after the iv administration of 100 IU hCG to pseudopregnant rabbits, the AC system of CL has become totally and irreversibly refractory to LH (3), progesterone secretion is terminated (6), luteal weight is reduced 50% (6, 7), and CL are morphologically regressing (7, 8). Concurrently in the ovary and as a result of the hCG administration, follicles ovulate within 12 h of the hCG injection and differentiate into CL (3, 8). When ovulation is induced by hCG during pregnancy, these new CL may resume the steroidogenic function lost by the original group of regressed CL, since pregnancy is often (3) but not always (7) maintained. However, in rats, the long range effect of an ovulatory/luteolytic dose of hCG on CL function is less clear (9, 10). We have shown

Received September 25, 1978.

Address all correspondence and requests for reprints to: Dr. Mary Hunzicker-Dunn, Department of Biochemistry, The Medical and Dental Schools, Northwestern University, Ward Memorial Building, 303 East Chicago Avenue, Chicago, Illinois 60611.

* This work was supported in part by NIH Grants HD-11356, HD-09581, and HD-07495.

that within 24 h of the administration of hCG (50 IU, sc; 50 IU, ip) to PMS gonadotropin (PMSG)-primed rats, pseudopregnant rats, and pregnant rats, the luteal AC system has become totally unresponsive to LH (3). In addition, we (3) and others (for review see Ref. 11) have observed that concurrently new ovulations occur, also within 24 h of the hCG (3). A recent report in the literature by Conti *et al.* (4) demonstrated that within 5–7 days after the administration of 200 IU hCG to PMSG- and hCG-primed rats containing heavily luteinized ovaries, the AC system, as seen in total ovarian homogenates, recovers its responsiveness to LH. In view of our previous findings that AC desensitization precedes irreversible luteolysis in rabbits and that desensitizing doses of hCG induce new ovulations within 24 h in both rabbits and rats, we wondered whether the reversal of desensitization seen in homogenates of total ovaries was indeed true reversal of the AC system of desensitized CL or whether it might be due to induction of fresh ovulations, formation of new CL and, possibly, development of additional responsive structures. Therefore, we investigated the effects of various doses of hCG on the morphological appearance and functional responsiveness of whole ovaries as well as of CL dissected from the ovaries of PMSG- and hCG-primed rats. The treatment schedule followed to prime immature rats with PMSG and cause ovulation with hCG was that used by Conti *et al.* (4), *i.e.* one that leads to a luteinized ovary showing reversal of desensitization. We also characterized the development of the hormonally responsive ovarian AC system after administration of hCG to PMSG-treated immature rats.

Materials and Methods

Female rats (Charles River, CD outbred; received at 21 days of age and weaned upon arrival, unless otherwise specified) were housed in air-conditioned quarters and allowed free access to water and a commercially pelleted food. Animals were subjected to the experimental design outlined in Fig. 1. Thus, 50 IU PMSG (Sigma Chemical Co., St. Louis, MO) dissolved in 0.5 ml 0.9% NaCl were injected sc into 252 25-day-old rats (1600 h on day 25). Sixty-five hours later (0900 h on day 28), rats were injected sc with a first ovulating dose (50 IU) of hCG (Ayerst Laboratories, Rouses Point, NY) dissolved in 0.5 ml 0.9% NaCl. Some rats were also injected at 33 days of age (0900 h) with a second desensitizing dose of hCG, consisting either of 200 IU sc (72 rats) or 50 IU sc (15 rats), or of 75 IU into the tail vein under light ether anesthesia (120 rats). Rats were killed by decapitation at the times indicated in Fig. 1 and in the text. Blood was collected from the trunk and allowed to clot overnight at 4 C. Serum was separated by centrifugation and saved at −20 C for subsequent progesterone determinations. One whole ovary, free of adhering connective tissue and bursa, or follicles and/or CL dissected from one ovary under a dissecting microscope were immediately placed in iced Krebs-Ringer bicarbonate buffer. The second ovary from some rats was either

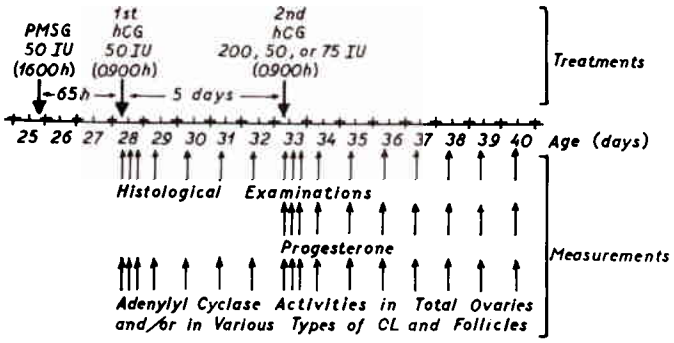

FIG. 1. Summary of treatment schedules and type and timing of measurements carried out. For details, see *Materials and Methods* and text.

treated identically or was prepared for microscopic examination by being fixed in Bouin's solution, embedded in paraffin, cut into 5-μm thick sections, and stained with hematoxylin-eosin.

Tissues were maintained in iced Krebs-Ringer bicarbonate buffer until dissections were completed. They were then homogenized in 10 vol 27% (wt/wt) sucrose, 10 mM Tris-HCl, and 1 mM EDTA, pH 7.5, and diluted with an equal volume of homogenization medium. Aliquots (20 μl) of the homogenates were then assayed for AC activity, as previously described (1, 2), in either the absence or presence of 10 μg/ml bovine serum albumin, 10 μg/ml LH (NIH-LH-B9, a generous gift of the Pituitary Hormone Distribution Program, NIAMDD), 20 μg/ml epinephrine (Parke-Davis, Detroit, MI), or 10 mM NaF. A second group of rats (Sprague-Dawley) were treated sc at 25 days of age with 15 IU PMSG dissolved in 0.1 ml 0.9% NaCl. Forty-eight hours later, rats were sacrificed. Ovaries were homogenized and membranes were prepared according to the method of Mintz *et al.* (12).

Progesterone levels were determined on tissue homogenates and serum samples extracted twice with petroleum ether and without subsequent chromatography (13) using a RIA based on antiserum 337 provided by Dr. G. D. Niswender and validated by Gibori and Richards (14) as modified by Day and Birnbaumer (13). Protein was determined by the procedure of Lowry *et al.* (15) using crystalline bovine serum albumin as standard.

Results

Effects of 50 IU PMSG, sc, on day 25 and of 50 IU hCG, sc, 65 h later on day 28

We characterized the morphology and hormonal responsiveness of intact rat ovaries as well as of ovarian follicles and CL before and after a first injection of 50 IU hCG (65 h after PMSG, at 0900 h on day 28) to the PMSG-primed rats.

By 65 h after PMSG injection, *i.e.* the time of hCG administration, the rat ovary already contained luteinized tissue. Ovulation points were not discernable and histological examination revealed that the luteal tissue consisted of follicles exhibiting various degrees of luteinization and containing entrapped ova (not shown). Preo-

vulatory follicles were also present. The AC of these ovaries was stimulated 2.5-fold by LH, 4.5-fold by epinephrine, and 10-fold by NaF (Fig. 2). The presence of a highly epinephrine-responsive AC system indicated on a biochemical basis the presence of luteal tissue at this stage of hormonal treatment, since previous studies have shown the almost complete absence of catecholamine-responsive AC in preovulatory follicles of rabbits and pigs (Hunzicker-Dunn, M., unpublished observations) (16). Using an injection regime which did not promote premature luteinization, we determined that follicule-predominated ovarian tissue from PMSG-primed rats also lacks significant catecholamine-responsive AC while exhibiting a highly LH-responsive AC (Table 1). Variations in AC conditions, namely high and low ATP concentrations and the presence of GTP, did not significantly enhance the catecholamine-response AC in this follicle-dominated preparation. This latter study, then, confirms the relative absence of a catecholamine-sensitive AC in follicular tissue.

The injection of hCG (50 IU, sc, 65 h after PMSG) promoted a rapid drop in LH-stimulated AC activity (Fig. 2), such that within 6 h, the ovarian AC system had become essentially unresponsive to LH. Ovarian LH-sensitive AC remained low for 3 days and then sharply increased, such that by 5 days after hCG administration (33-day-old rats), ovarian AC was stimulated 6- to 9-fold by LH. Epinephrine- and NaF-stimulated ovarian AC activities also increased by 2 days after the first hCG injection; however, the patterns of responsiveness of these two agonists were different (Fig. 2). Visual inspection of the ovaries revealed that within 2 days after the first hCG treatment, approximately nine new ovulation points were present per ovary. However, by day 4, the new CL could not be distinguished from the original luteal tissue present at the time of the hCG injection. Thus, dissection and subsequent AC assay of individual luteal tissues did not reveal significant differences among CL (not shown). Histological examination of the ovaries in PMSG- and hCG-primed rats on day 33 revealed the uniform presence of solid CL as well as preantral and early small antral follicles (Fig. 3).

TABLE 1. Responsiveness of PMSG-primed rat ovarian membrane AC to LH and isoproterenol

Additions[a]	AC activities			
	Minus GTP		Plus GTP[a]	
	Absolute (pmol cAMP/mg·min)	Relative (-fold)	Absolute (pmoles cAMP/mg·min)	Relative (-fold)
0.10 mM ATP				
None	6.6 ± 0.2		16.4 ± 0.2	
LH	22.5 ± 0.3	3.41	68.9 ± 2.5	4.20
Isoproterenol	8.2 ± 0.3	1.24	36.1 ± 1.5	2.20
2.87 mM ATP				
None	22.0 ± 1.9		56.3 ± 4.1	
LH	129.3 ± 4.6	5.88	356.2 ± 17.1	6.33
Isoproterenol	33.4 ± 3.7	1.52	108.9 ± 4.0	1.93

Rats (Sprague-Dawley) were treated at 23 days of age with 15 IU PMSG in 0.1 ml 0.9% NaCl (sc) and sacrificed 48 h later. Ovaries were homogenized and membranes were prepared according to Mintz *et al.* (12).

[a] Assay conditions were those described in *Materials and Methods*, except that the final concentration of ATP was varied as indicated. When present, the LH concentration was 10 μg/ml, isoproterenol was 10^{-4} M, and GTP was 10^{-5} M. Membrane protein was 11 μg/assay. Values represent the mean ± SD of triplicate determinations.

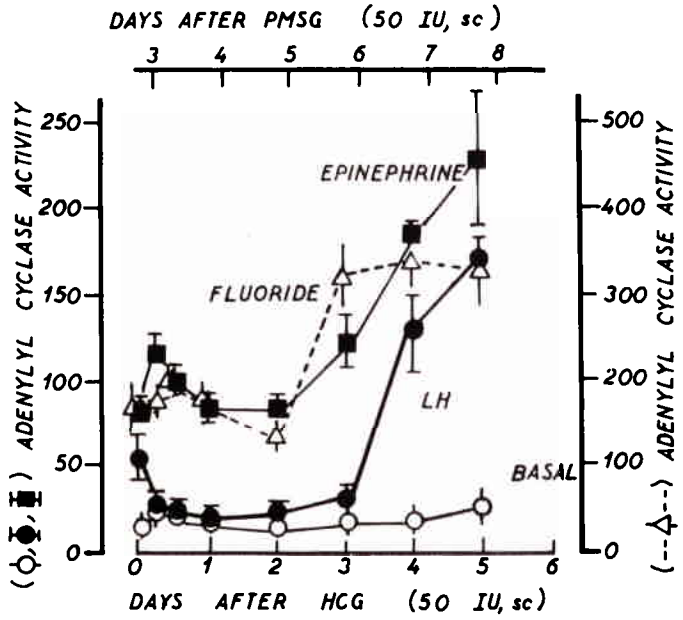

FIG. 2. Effect of the sc administration of 50 IU hCG to PMSG-primed rats on the responsiveness of the AC system to LH, epinephrine, and NaF. Twenty-five-day-old rats received 50 IU PMSG, sc, at 1600 h and 50 IU hCG, sc, 65 h later. Rats were sacrificed at the times indicated on the figure, and ovaries were cleaned of extraneous tissue and homogenized, as described in *Materials and Methods*. AC activities were determined in homogenates of ovaries in the absence (○) or presence of 10 μg/ml LH (●), 20 μg/ml epinephrine (■), or 10 mM NaF (Δ). In each experiment, triplicate determinations were made on one ovary from a single rat. The mean ± SEM are shown where two or three such assays were performed.

Effect of 200 IU hCG, sc, on day 33

We injected 200 IU hCG, sc, to 33-day-old rats primed with PMSG and hCG, as described above, and determined its effect(s) on ovarian AC, ovarian histology, and serum progesterone levels.

As described above, the AC system in the whole ovary before the second hCG injection was highly responsive to LH, epinephrine, and NaF (Fig. 2). Upon sc injection of 200 IU hCG, LH-stimulated AC activity rapidly de-

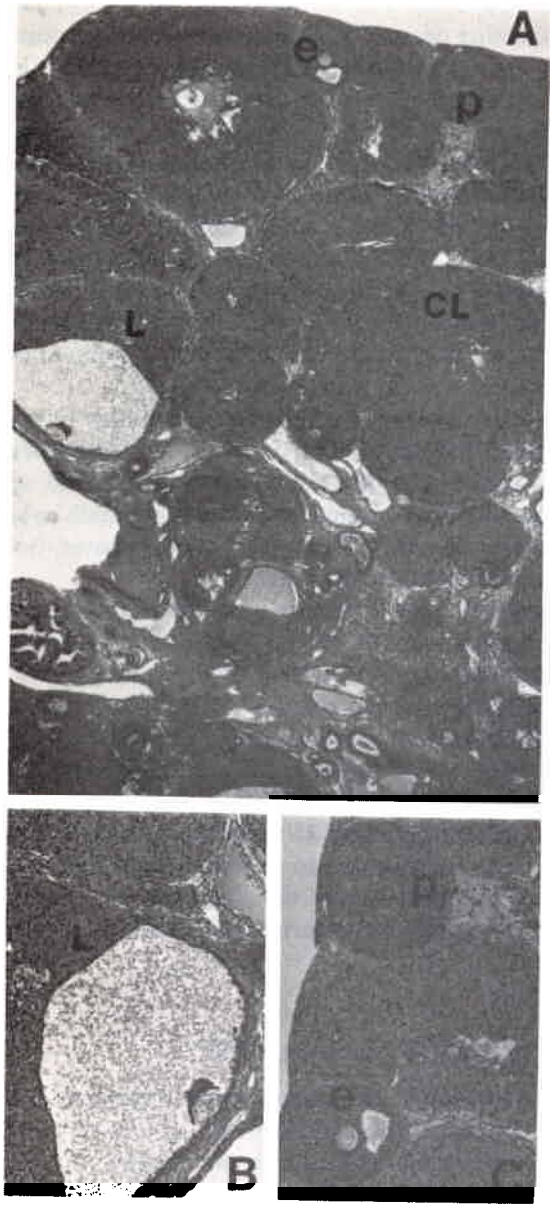

FIG. 3. Photomicrographs of ovarian structures in rats primed with 50 IU PMSG on day 25 of age, injected with 50 IU hCG 65 h afterwards, and sacrificed at 33 days of age. A, ×90; B, ×190; C, ×190. L, Luteinized follicle; p, preantral follicle; e, early antral follicle.

clined, such that by 24 h, the ovarian AC was no longer stimulated by LH (Fig. 4). LH-stimulable AC remained low for 2 days after the injection of 200 IU hCG but then steadily increased during the next 4 days. During the initial time period after the injection of 200 IU hCG, both epinephrine- and NaF-stimulated ovarian AC activities were also reduced, but the decline was less substantial and more transient than the reduction in LH-stimulated AC activity. These changes in LH-stimulate AC agree closely with those reported by Conti *et al.* (4).

As an indicator of ovarian function, we measured serum progesterone levels after the injection of 200 IU hCG. Serum progesterone levels began to decline by 12 h after hCG administration and thereafter continued to decline steadily (Fig. 5). Thus, the apparent recovery of ovarian LH-stimulable AC was not accompanied by a recovery of luteal function, *i.e.* a concomitant return of serum progesterone levels to control values. In contrast to serum progesterone levels, which declined after the desensitizing dose of hCG, progesterone content in ovarian homogenates did not vary significantly at any of the observed times after the injection of 200 IU of hCG from a value of 61.0 ± 8.8 pg progesterone/μg ovarian protein, as determined in 33-day-old PMSG- and hCG-primed rats before the second hCG injection.

To determine the morphological changes which occur in PMSG- and hCG-primed rat ovaries after the administration of a desensitizing dose of hCG (200 IU, sc), we inspected the ovaries of these rats both macroscopically (under a dissecting microscope) and histologically. Macroscopic observation revealed the presence within 2 days after injection of 200 IU hCG of distinct ovulation points. By day 5 after hCG administration, two sets of CL, one vascular and the other less vascular, could be distinguished even without the aid of a dissecting microscope. Thus, both ovulation and formation of new CL had occurred in response to 200 IU hCG. Histological observation revealed that administration of the 200-IU dose of

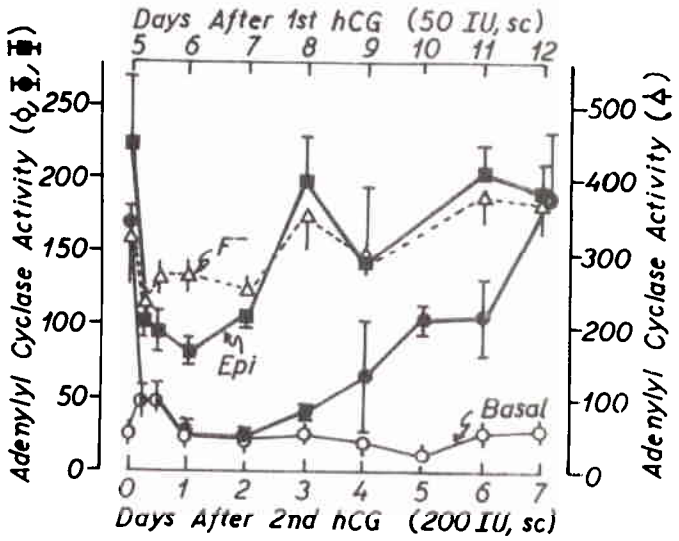

FIG. 4. Effect of the sc administration of 200 IU hCG to PMSG- and hCG-primed rats on the responsiveness of the AC system to LH, epinephrine, and NaF. Immature female rats received 50 IU PMSG on day 25, 50 IU hCG on day 28, and 200 IU of hCG on day 33, as shown on Fig. 1 and detailed in *Materials and Methods*. Rats were sacrificed after the 200 IU hCG injection at the times indicated on the figure, and AC activity was measured in ovarian homogenates. For further details, see Fig. 2. In each experiment, triplicate determinations of AC activity were made on both ovaries from a single rat. The mean ± SEM are shown where three or four such assays were performed.

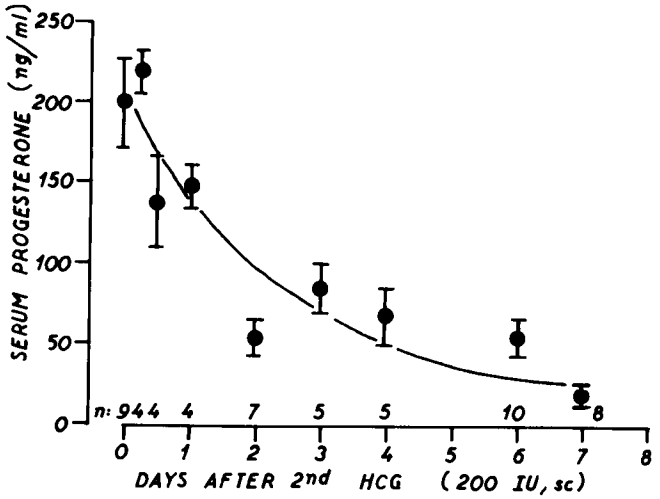

FIG. 5. Effect of administration of 200 IU hCG to PMSG- and hCG-primed rats on serum progesterone levels. The injection regime is as described in Fig. 4. Blood was collected from rats upon decapitation, serum was separated from clotted blood the following morning, and progesterone levels were determined in aliquots of the serum by RIA using the progesterone antibody no. 337 supplied by Dr. G. Niswender, as described in *Materials and Methods.*

hCG led to ovaries that contained by day 2 a fair number of large antral follicles, some quite large (Fig. 6, A1–A3). By day 3, it was observed that the structures with fresh ovulation points on the ovarian surface contained a substantial number of lutein cells (Fig. 6, B1 and B2). By days 4 and 5, most of the ovarian mass could be considered luteinized, although many of the CL-like structures (luteinized follicles, structures with variable sized cavities in the center) were not of a normal type (Fig. 6C). In some instances, it was possible to distinguish between older and newer types of luteinized structures, since some of them showed degenerative changes such as those described by Spies *et al.* (17) and others (18, 19). By 7 days, degenerative changes were clearly associated with one type of CL; lutein cells were irregular in shape, cytoplasm was vacuolated, nuclei were pycnotic or fragmenting (Fig. 6, D2 and D4, and Fig. 7, B1 and B2), and the boundary between the CL and surrounding tissue was disappearing. No such degenerative changes were visible in another group of CL; lutein cells were more regular in shape and distribution, nuclei were more centrally located (Fig. 6, D1 and D3, and Fig. 7, A1 and A2), and the boundary between CL and surrounding tissue was distinct. Thus, the administration of 200 IU hCG to PMSG- and hCG-primed rats resulted in regression of the original set of CL, development of small antral follicles to an ovulable state, followed by ovulation, and the subsequent formation of a new set of CL as well as of luteinized follicles.

To determine the specific ovarian tissue responsible for the increased LH responsiveness of the AC system demonstrable in the whole ovary by 7 days after the desensitizing dose of hCG, we assayed both groups of CL for AC activity. Assay of homogenates of CL dissected from ovaries 7 days after desensitization of luteal AC induced by 200 IU hCG revealed that the original CL (with poorly defined edges) remained poorly responsive to LH stimulation, while the newly formed CL (induced by 200 IU hCG; with easily defined edges) achieved better than a 6-fold stimulation by LH (Table 2). It is noteworthy that while LH responsiveness in the original CL was nearly completely abolished, the responsiveness of AC to both epinephrine and NaF was much less affected.

Analysis of progesterone content in the two macroscopically distinguishable types of CL 7 days after 200 IU hCG showed a small difference between old and new CL; the new CL contained 1.83 times as much progesterone as the old ones (Table 2). This difference, however, was not statistically significant.

Ovarian weights did not increase during the 7 days after the injection of 200 IU hCG even though new CL were forming (mean ± SEM of ovarian weights of a pair of ovaries from three animals for 0, 1, 3, 5, and 7 days post-hCG injection were, respectively, 180.3 ± 13.8, 228.3 ± 1.8, 196.6 ± 31.8, 148.6 ± 7.3, and 164.4 ± 20.7 mg). In fact, by day 5, ovarian weights were reduced compared to the weights before hCG treatment. Since luteal tissue is the main contributor to ovarian weight, these results indirectly indicate that the original CL loose tissue weight, a known characteristic of luteal regression (6, 7, 20–23).

Effect of 50 IU hCG, sc, on day 33

We determined the effect of the administration of a lower concentration of hCG (50 IU) sc to 33-day-old PMSG- and hCG-primed rats on ovarian AC and morphology 4 and 7 days after the hormone injection.

At the time of the hCG injection, the AC in dissected CL was highly responsive to LH, epinephrine, and NaF (Table 3). Macroscopic inspection of the ovaries 4 and 7 days after hCG injection revealed the absence of new ovulation points. Thus, only a single group of CL was present. Assay of luteal AC activity revealed that the LH-stimulated activity was greatly reduced by 4 days after the injection of 50 IU hCG and remained low 7 days post-hCG injection. Epinephrine- and NaF-stimulable AC activities were also markedly reduced but, as observed after the injection of 200 IU hCG, not to the same extent as the LH-stimulable AC activity. Thus, although the sc injection of 50 IU hCG to 33-day-old rats promoted desensitization of the luteal AC to LH as well as partial desensitization to epinephrine and NaF, this concentration was not sufficient to induce new ovulations.

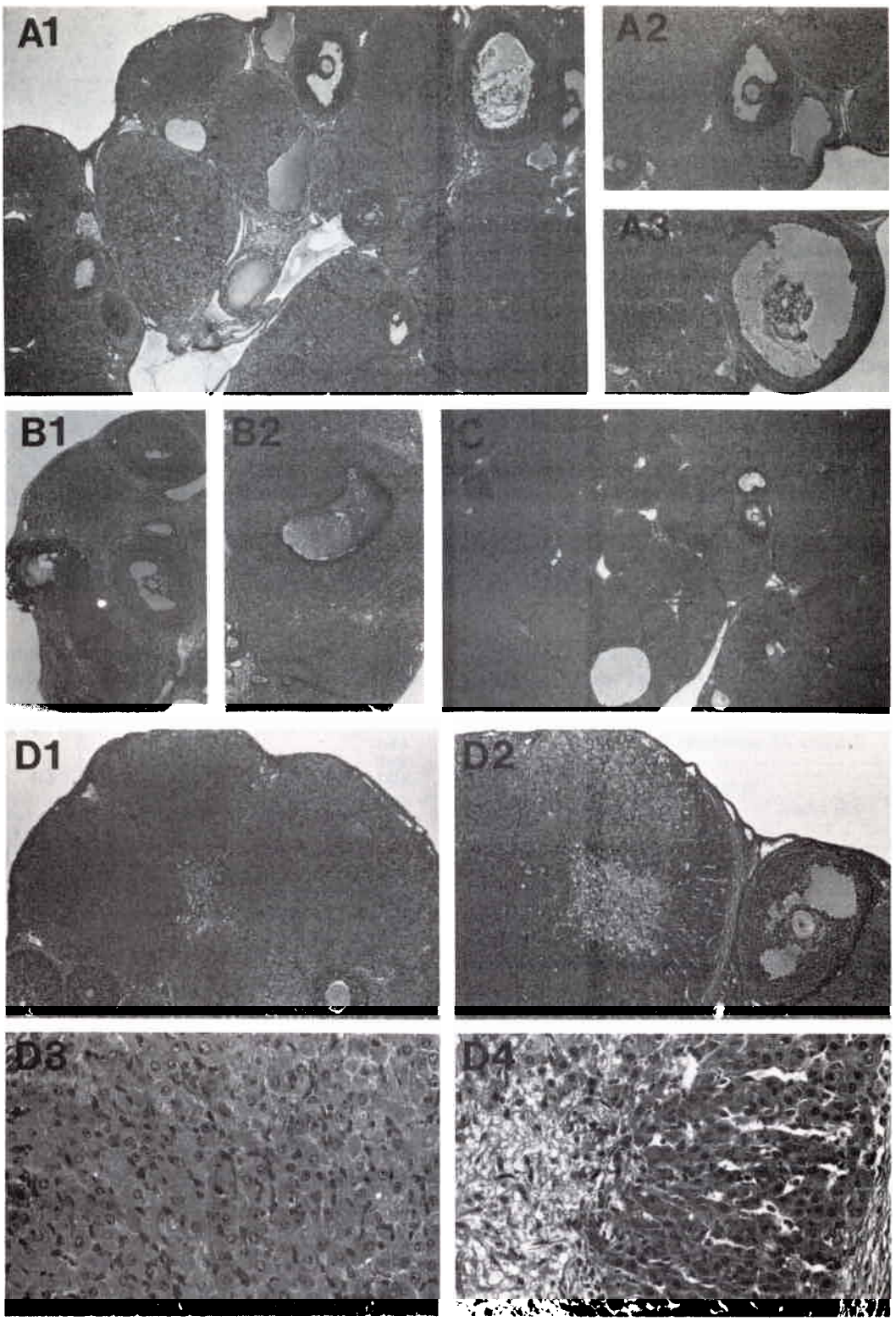

FIG. 6. Photomicrographs of ovaries from PMSG- and hCG-treated rats at various times after receiving a second dose of 200 IU hCG. For details, see Fig. 1 and *Materials and Methods*. A, Ovaries taken 2 days after the second injection of hCG; A1, general view (×90); A2, antral follicle (×115); A3, large antral follicles with ovum (×115). B, Luteinized follicles with large ovulation point 3 days after the second injection of hCG; B1, ovulation point and partially luteinized granulosa cells (×140); B2, area with fully luteinized granulosa cells from same follicle (×140). C, Luteinized ovary 5 days after the second injection of hCG (×65). D, Old and new CL lutea from ovaries 7 days after the second injection of hCG; D1, low powered view of area with mostly new CL (×270); D2, same area with old CL (×270); D3, higher magnification view of D1 (×600); D4, higher magnification view of D2 (×600).

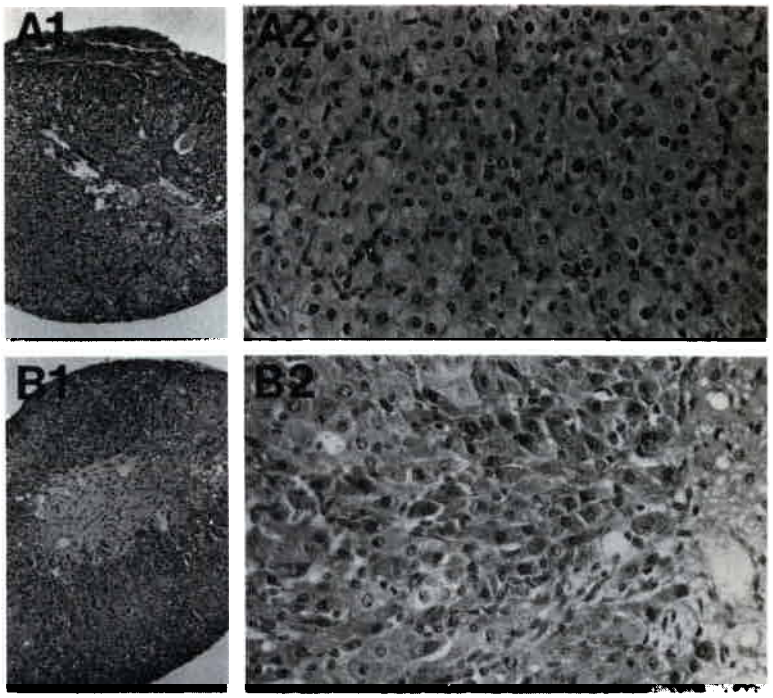

FIG. 7. Photomicrographs of CL dissected 7 days after the second (desensitizing) injection of hCG (200 IU, sc). For details, see Fig. 1, *Materials and Methods*, and text. A, New CL apparently formed after the second hCG injection. Macroscopically, CL of this type were relatively large, had a relatively less vascular appearance, and contained an LH-responsive AC system (Table 2). A1, Low power view (×180); A2, higher magnification of CL shown in A1 (×640). B, Old CL apparently formed before hCG injection. Macroscopically, CL of this type had a more vascularized appearance, were relatively small, and contained AC systems that did not respond to LH (Table 2). B1, Low power view (×180); B2, higher magnification of CL shown in B1 (×640).

TABLE 2. Effect of the administration of 200 IU hCG, sc, to PMSG- and hCG-primed rats on luteal AC activity and luteal progesterone content

Days after 2nd hCG	Relative AC stimulation[a]				Progesterone[c] (pg/μg luteal protein)
	Tissue[b]	LH (-fold)	Epinephrine (-fold)	NaF (-fold)	
0	CL	7.9 ± 1.9	8.7 ± 0.8	17.3 ± 1.5	
7	Old CL	1.8 ± 0.2	5.5 ± 0.5	9.1 ± 0.9	179 ± 17
7	New CL	6.4 ± 0.1	9.1 ± 0.7	13.9 ± 3.7	327 ± 54

hCG (200 IU) was administered sc in 0.5 ml 0.9% NaCl at 0900 h to 33-day-old rats primed with PMSG, as described in *Materials and Methods* and outlined in Fig. 1.

[a] Values are the mean ± SEM of assays, in each of which CL from two rats were used. For CL at time 0 and new CL from ovaries obtained 7 days post-hCG, n = 3; for old CL from ovaries 7 days post-hCG, n = 5. Relative stimulation of AC is calculated by dividing hormonally stimulated activity by basal activity.

[b] On day 0, CL were indistinguishable among themselves. On day 7, two groups of CL were present. Old CL had a more vascular appearance, were smaller, and exhibited indistinct edges adjacent to the surrounding tissue; new CL appeared less vascular, were larger, and exhibited clearly defined edges.

[c] Progesterone was assayed in separate aliquots of the same homogenates assayed for AC activities. Values represent the mean ± SEM of the number of experiments indicated under Footnote [a].

TABLE 3. Effect of the administration of 50 IU hCG, sc, to PMSG- and hCG-primed immature rats on luteal AC activity

Days after 2nd hCG	Absolute activities (pmol cAMP/mg protein·min)[a]				Relative stimulation (-fold)[b]		
	Basal	LH	Epinephrine	NaF	LH	Epinephrine	NaF
0	26 ± 1	191 ± 43	226 ± 13	451 ± 25	7.4 ± 1.9	8.7 ± 0.8	17.3 ± 1.5
4	5 ± 1	17 ± 7	42 ± 11	71 ± 4	2.7 ± 0.8	6.3 ± 0.3	15.0 ± 4.0
7	8 ± 2	12 ± 2	56 ± 17	88 ± 27	1.9 ± 0.5	7.0 ± 0.8	11.4 ± 1.8

hCG (50 IU) was administered sc in 0.5 ml 0.9% NaCl at 0900 h to 33-day-old rats primed with PMSG at 1600 h on day 25 and with hCG 65 h later, as described in *Materials and Methods* and outlined in Fig. 1. Ovarian CL were indistinguishable among themselves.

[a] Values are the mean ± SEM of assays, in each of which CL from two rats were used. For 0 and 4 days, n = 3; for 7 days, n = 4.

[b] Relative stimulation of AC is calculated by dividing hormonally or fluoride-stimulated activities by basal AC activity. Values represent the mean ± SEM of the ratios.

Effect of 75 IU hCG, iv, on day 33

We determined the effect of the administration of 75 IU hCG injected iv rather than sc to 33-day-old PMSG- and hCG-primed rats on ovarian AC and morphology. This injection regime should promote simultaneous ovulation of all mature preovulatory follicles present in these heavily luteinized ovaries at the time of the hCG injection. Macroscopic inspection of the ovaries revealed that the iv injection of 75 IU hCG promoted no new ovulations, indicating the absence of mature ovulable follicles in these ovaries. These results confirm our earlier conclusions that follicles of preovulatory size are not present in these heavily luteinized ovaries and that only smaller less mature follicles are present. Furthermore, when these smaller follicles are subjected to prolonged elevations in hCG levels, such as occurs with sc injection of 200 IU hCG, they mature to a preovulatory size and ovulate. The source of the stimulus for ovulation is not clear at

this time. Possibly, increasing estrogen levels override the inhibitory influence of progesterone on the hypothalamic-pituitary axis to induce an endogenous LH surge.

Assay of AC activity revealed that within 6 h of the iv injection of hCG, LH-stimulated AC activity in dissected CL was greatly reduced and the AC system was no longer responsive to LH stimulation (Fig. 8). By 24 h, LH-stimulated AC activity was further reduced to preinjection basal AC levels. During the subsequent 7 days, dissected luteal AC remained unresponsive to LH stimulation.

The initial decline in epinephrine- and NaF-stimulated AC activities after the iv injection of 75 IU hCG closely resembled a similar decline seen after sc administration of 200 IU hCG. However, the recovery of epinephrine- as well as NaF-responsive AC activity, apparent after the injection of 200 IU hCG, was markedly retarded after the injection of 75 IU hCG.

Rather than promoting ovulation, the iv injection of 75 IU hCG promoted marked follicular proliferation. There was a significant increase in the number of small antral follicles seen throughout the ovary as cords of follicles between CL (not shown). However, the follicles tended to luteinize (becoming atretic?) before they reached preovulatory size. Assay of these ovarian structures (small antral follicles and luteinized follicles) as well as of the whole ovary for AC activity during the 7 days after hCG injection resulted in values for all three tissues which were not significantly different from those presented in Fig. 8; none of these tissues acquired an LH-responsive AC system. However, by 12 days after the injection of 75 IU hCG, two sets of CL were discernible, one formed of CL with fresh ovulation points and having a responsive LH-stimulable AC (AC activity in picomoles of cAMP per mg protein/min: basal, 20; LH, 166; epinephrine, 296; and NaF, 353) and the other consisting of CL with an AC which was not responsive to LH (AC activities: basal, 34; LH, 22; epinephrine, 148; and NaF, 389). More than likely, the normal cyclic ovarian behavior patterns had by then been initiated in these rats.

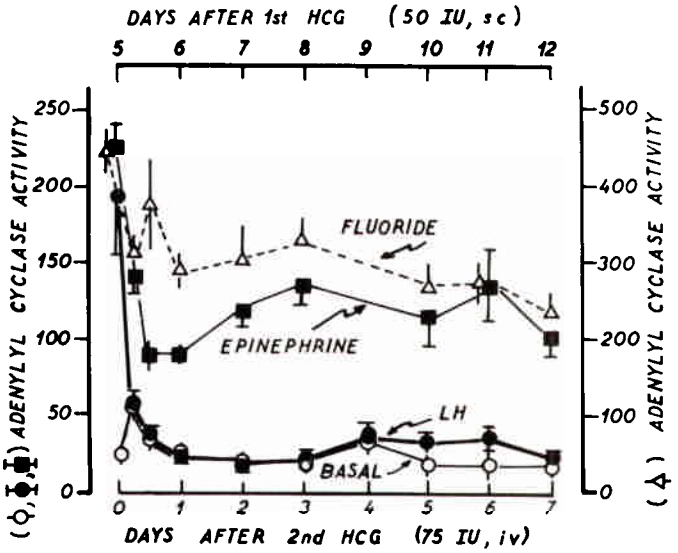

FIG. 8. Effect of the iv administration of 75 IU hCG to PMSG- and hCG-primed rats on the responsiveness of the AC system to LH, epinephrine, and NaF. Gonadotrophin priming was as described in *Materials and Methods* and shown in Fig. 1. Seventy-five international units of hCG were administered iv to 33-day-old rats at 0900 h, CL were dissected from ovaries obtained from rats at the times indicated, and AC activities were measured in luteal homogenates, as described in *Materials and Methods*. In each experiment, triplicate determinations of AC activities were made on CL from both ovaries of a single rat. The mean ± SEM are shown where two or three such assays were performed.

Discussion

Heavily luteinized ovaries obtained by priming immature rats with PMSG and administering hCG 65 h later, such as was done in the studies reported above, have recently been used to explore the biochemical consequences of a subsequent (desensitizing) dose of hCG, both in terms of changes in hormone-stimulated AC activities and in terms of alterations in steroidogenic activity of cells isolated from such treated ovaries (4, 24, 25). As mentioned earlier in this paper, the apparent return in LH-stimulable AC in the face of the known luteolytic and ovulatory actions of hCG coupled to our own previous findings that hCG-induced desensitization of AC in rabbit CL persists for at least 14 days (3) lead us to explore certain aspects of morphological and functional changes as well as actual cellular localization of the modifications of AC activities that occur in response to desensitizing doses of hCG in PMSG- and hCG-primed rat ovaries. As shown by the data presented above, ovaries primed and caused to luteinize in this manner contain not only luteal tissue but also many large preantral and small antral follicles. Injection of hCG results not only in luteolysis and desensitization but also, and importantly so, in growth and development of small antral follicles to preovulatory follicles which, 2–3 days afterwards, luteinized without (becoming atretic?) or with ovulation, depending on the dose of hCG used. Thus, ovaries from these PMSG- and hCG-primed rats subjected to a second (desensitizing) dose of hCG cannot be considered as being composed mostly of a single type of luteal tissue; rather, they are a heterogeneous mixture of cell types, including granulosa and theca cells from preantral and antral follicles and luteal cells from old regressing and new forming CL, all at varying stages of development depending on the time of observation after the second hCG injection.

It follows from these findings that extreme caution should be exerted when interpreting data obtained either

by analyzing activities in homogenates of whole ovaries (4) or by evaluating steroidogenic properties of cells derived by enzymatic digestion of hCG-treated luteinized ovaries without separation of cell types (24, 25). For example, our findings, obtained through analysis of AC activities in homogenates of dissected CL (Fig. 8 and Table 2), clearly indicate that the desensitization of luteal AC to LH is permanent in the rat. This conclusion is in sharp contrast to what has been (4) or would be (Fig. 2) concluded from the simple analysis of whole ovary homogenates.

It is commonly assumed that ovaries of PMSG-primed hCG-ovulated rats contain only the smallest of preantral follicles. Our finding of so many large preantral follicles in ovaries 5 days after administration of the first (luteinizing) dose of hCG may be related to the timing of the first hCG injection. Quinn and Farrow (26) clearly showed that PMSG treatment of 24-day-old immature rats results 55–56 h afterwards in a critical period, *i.e.* in an endogenous surge of a normal ovulatory complement of pituitary gonadotropins. Injection of exogenous hCG 65 h (4) after the PMSG injection may thus act as a late comer, so that rather than causing a synchronous massive ovulation and luteinization it may affect postively (ovulating and luteinizing) only those follicles that had not been sufficiently mature to respond to the endogenous ovulatory hormones 10 h earlier but which had subsequently developed the necessary responsiveness. While this type of sequential treatment leads to a heavily luteinized ovary, it obviously must also allow for initiation of maturation of new follicles, which in all likelihood are the ones that in our studies show up 5 days later as large preantral and small antral follicles and, after sc treatment with high doses of hCG, mature (develop large antra), ovulate, and luteinize to give new CL.

Of interest also was the finding that newly formed CL, although acquiring an LH-stimulable AC system, did not become functionally active, as seen by the low serum progesterone levels 7 days after 200 IU hCG. This is in contrast to the marked activity exhibited by the original luteal tissues which had become active 5–6 days after gonadotropin-induced luteinization (endogenous gonadotropins surge on day 27 and exogenous hCG surges on day 28). We are currently investigating whether this lack of CL activation is related to a possible absence of PRL after the treatment with 200 IU hCG, or whether some additional factors important in CL function are absent.

While administration of any of the desensitizing concentrations of hCG caused the AC in the heavily luteinized ovaries to become permanently refractory to LH, only transient drops in epinephrine- and NaF-responsive AC activity occurred. In no instance that we tested did the luteal AC exhibit a total refractoriness to epinephrine or NaF. These data on NaF are not surprising, since NaF is believed to interact directly with the catalytic subuni of the AC system and, therefore, does not require a receptor. However, epinephrine activation of the AC system is a receptor-mediated event. Since the physiological significance of epinephrine in CL function is not presently known, the physiological basis for CL maintaining an epinephrine-responsive AC when they are no longer steroidogenically active and are, in fact, regressing is an enigma. Further investigations are clearly required to unravel this phenomenon.

In summary, we have characterized in PMSG-primed rats the development of the LH-stimulable AC in luteal tissue when hCG (50 IU, sc) is injected 65 h after PMSG. Furthermore, we have established that this treatment schedule results in ovaries that, upon administration of large doses of hCG 5 days afterwards, respond not only with a luteolytic reaction and permanent desensitization of AC to LH stimulation but also by developing the large antral follicles which ovulate and form new CL.

References

1. Hunzicker-Dunn, M., and L. Birnbaumer, Adenylyl cyclase activities in ovarian tissues. II. Regulation of responsiveness to LH, FSH, and PGE_1 in the rabbit, *Endocrinology* **99:** 185, 1976.
2. Hunzicker-Dunn, M., and L. Birnbaumer, Adenylyl cyclase activities in ovarian tissues. III. Regulation of responsiveness to LH, FSH, and PGE_1 in the prepubertal, cycling, pregnant, and pseudopregnant rat, *Endocrinology* **99:** 198, 1976.
3. Hunzicker-Dunn, M., and L. Birnbaumer, Adenylyl cyclase activities in ovarian tissues. IV. Gonadotropin-induced desensitization of the luteal adenylyl cyclase throughout pregnancy and pseudopregnancy in the rabbit and rat, *Endocrinology* **99:** 211, 1976.
4. Conti, M., J. P. Harwood, A. J. W. Hsueh, M. L. Dufau, and K. J. Catt, Gonadotrophin-induced loss of hormone receptors and desensitization of adenylate cyclase in the ovary, *J Biol Chem* **251:** 7729, 1976.
5. Conti, M., J. P. Harwood, M. L. Dufau, and K. J. Catt, Regulation of luteinizing hormone receptors and adenylate cyclase activity by gonadotrophin in the rat ovary, *Mol Pharmacol* **13:** 1024, 1977.
6. Flint, A. P. F., D. L. Grinwich, T. G. Kennedy, and D. T. Armstrong, Metabolism of the corpus luteum during luteolysis in the pseudopregnant rabbit, *Endocrinology* **94:** 509, 1974.
7. Stormshak, F., and L. E. Casida, Effect of gonadotropins on corpora lutea of pseudopregnant rabbits, *Endocrinology* **75:** 321, 1964.
8. Spies, H. G., L. L. Coon, and H. T. Gier, Luteolytic effect of LH and hCG on the corpora lutea of pseudopregnant rabbits, *Endocrinology* **78:** 67, 1966.
9. Rothchild, I., Interrelations between progesterone and the ovary, pituitary, and central nervous system in the control of ovulation and the regulation of progesterone secretion, *Vitam Horm* **23:** 209, 1965.
10. Banik, U. L., Pregnancy-terminating effect of human chorionic gonadotropin in rats, *J Reprod Fertil* **42:** 67, 1975.
11. Hunzicker-Dunn, M., J. Bockaert, and L. Birnbaumer, Physiological aspects of appearance and desensitization of gonadotropin-sensitive adenylyl cyclase in ovarian tissues and membranes of rabbits in rats and pigs, *In* O'Malley, B. W., and L. Birnbaumer (eds.), Receptors and Hormone Action, vol. 3, Academic Press, New York, 1978, p. 393.
12. Mintz, Y., Y. Amir, A. Amsterdam, H. R. Lindner, and Y. Saloman, Adenylate cyclase activity in purified membranes from rat ovary *Mol Cell Endocrinol* **11:** 265, 1978.
13. Day, S. L., and L. Birnbaumer, Protection by prolactin of rat corpus luteum function and adenylyl cyclase stimulability after an estradiol

benzoate-induced ovulatory (desensitizing) surge of luteinizing hormone, *Endocrinology*, in press.
14. Gibori, G., and J. S. Richards, Dissociation of two distinct luteotropic events of prolactin: regulation of luteinizing hormone-receptor content and progesterone secretion during pregnancy, *Endocrinology* **102:** 767, 1978.
15. Lowry, D. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall, Protein measurement with the Folin phenol reagent, *J Biol Chem* **193:** 265, 1951.
16. Birnbaumer, L., P.-C. Yang, M. Hunzicker-Dunn, J. Bockaert, and J. M. Duran, Adenylyl cyclase activity in ovarian tissues. I. Homogenization and conditions of assay in graafian follicles and corpora lutea of rabbits, rats and pits: regulation by ATP, and some comparative properties, *Endocrinology* **99:** 163, 1976.
17. Spies, H. G., J. Hilliard, and C. H. Sawyer, Maintenance of corpora lutea and pregnancy in hypophysectomized rabbits, *Endocrinology* **83:** 354, 1968.
18. Dott, H. M., Lysosomes and lysosomal enzymes in the reproductive tract, *In* Dingle, J. T., and H. B. Bell (eds.), Lysosomes in Biology and Pathology, North-Holland, Amsterdam, London, 1969, p. 330.
19. Koering, M. J., and M. J. Thor, Structural changes in the regressing corpus luteum of rabbit, *Biol Reprod* **17:** 719, 1978.
20. Gutknecht, G. D., G. W. Duncan, and L. J. Wyngarden, Inhibition of prostaglandin $F_{2\alpha}$ of LH induced luteolysis in the pseudopregnant rabbit by 17β-estradiol, *Proc Soc Exp Biol Med* **139:** 406, 1972.
21. Keyes, P. L., and A. V. Nalbandov, Maintenance and function of corpora lutea in rabbits depend on estrogen, *Endocrinology* **80:** 938, 1967.
22. Hilliard, J., H. G. Spies, and C. H. Sawyer, Hormonal factors regulating ovarian cholesterol mobilization and progestin secretion in intact and hypohysectomized rabbits, *In* McKerns, K. W. (ed.), The Gonads, Appleton-Century-Crofts, New York, 1969, p. 55.
23. Hilliard, J., H. G. Spies, and C. H. Sawyer, Cholesterol storage and progestin secretion during pregnancy and pseudopregnancy in the rabbit, *Endocrinology* **82:** 157, 1968.
24. Conti, M., J. P. Harwood, M. L. Dufau, and K. J. Catt, Effect of gonadotropin-induced receptor regulation on biological responses of isolated rat luteal cells, *J Biol Chem* **252:** 8869, 1977.
25. Catt, K. J., J. P. Harwood, N. D. Richert, P. M. Conn, M. Conti, and M. L. Dufau, Luteal desensitization: hormonal regulation of LH receptors, adenylate cyclase and steroidogenic responses in the luteal cell, *In* Channing, C. P., J. M. Marsh, and W. D. Sadler (eds.), Ovarian Follicular and Corpus Luteum Function, Plenum Press, New York, 1979, p. 647.
26. Quinn, D. L., and M. X. Zarrow, Inhibition of pregnant mare's serum-induced ovulation in the immature rat, *Endocrinology* **74:** 309, 1964.